Science Question 1: Methodological Considerations for Evaluating Epidemiologic Studies

Key Points

1. **Use of specified guidelines for assessing epidemiologic studies, also recommended by NRC, is absent from the preliminary materials document.**
2. **Four ecological studies in Table 2-9 "Evidence pertaining to cancer following oral exposure to Cr(VI)" are severely limited.**
3. **Occupational studies of Cr(VI) are of greater quality/utility and should be considered for Table 2-9.**

Mina Suh

ToxStrategies, Inc.

Supported by ACC

October 29, 2014

Aspects to Consider in Evaluating Epidemiologic Studies (NRC, 2014)

Epidemiologic Studies

- Documentation of study design, methods, population characteristics, and results.
- Definition and selection of the study group and comparison group.
- Ascertainment of exposure to the chemical or mixture.
- Ascertainment of disease or health effect.
- Duration of exposure and follow-up and adequacy for assessing the occurrence of effects.
- Characterization of exposure during critical periods.
- Participation rates and potential for selection bias as a result of the achieved participation rates.
- Measurement error…and other types of information bias.
- Potential confounding and other sources of bias addressed in the study design or in the analysis of results.

- **Absent from the tables:**
  - Characterization of maternal exposures, critical windows of susceptibility
  - Studies of qualitative, semi-quantitative exposure assessments- No discussion of potential measurement errors
  - No discussion of ecological bias/fallacy

Risk of Bias Assessments of Epidemiologic Studies (NRC, 2014)

Factors to Assess Risk of Bias in Observational Studies	Rationale
Confounding and selection	Difference in the distribution of risk factors between groups
Measurement error	Exposure, outcomes, or confounders are not measured correctly

Adapted from Table 5-1 (NRC, 2014)

- **Preliminary evidence tables present all studies as equal**
- **It is difficult to discuss methodological considerations (Section 1.2.4) including interval validity – Study-specific information is not complete or missing**
- **Guidelines that can be also considered: STROBE, GRADE, Cochrane Collaboration**

Case Study: Table 2-9 "Evidence Pertaining to Cancer Following Oral Exposure to Hexavalent Chromium"

- **Three studies evaluated 5 villages in China (Zhang and Li, 1997, Beaumont et al. 2008; Kerger et al. 2009)**
- **Linos et al. (2011) investigated residents in an industrial region of Greece**

Factors to Assess Risk of Bias	Study Details
Confounding and selection	Ecological in design with no individual data Confounding cannot be assessed. Differential distributions of extraneous factors between comparison groups are expected
Measurement error	Population-level exposures • Greeks do not typically drink municipal water • Cr(VI) in wells varied within any village of China. Residents also likely drank from municipal water.

Relevance of Occupational Studies of Cr(VI)

- **Discrepancy in study inclusion criteria?**
  - Ecological studies of oral exposures were used to evaluate cancer in Table 2-9
  - Occupational studies of inhalation exposures were used to evaluate gastrointestinal effects in Table 2-1 "Evidence pertaining to gastrointestinal (GI) effects following exposure to Cr(VI)"
- **Relevance of occupational studies for evaluating ingestion exposures**
  - With high exposure concentrations (workers in chromate production industry were exposed in the upper bounds of hundreds $\mu g/m^3$ Cr(VI) historically), mucocilliary clearance from the lung can lead to ingestion
  - Oral respiration is possible and hence the potential for ingestion
- **Several meta-analyses of occupational studies have been conducted evaluating GI effects from Cr(VI)**

Example: Occupational cohort studies in Table 2-1 (Birk et al. 2006, Hayes et al. 1979, Luippold et al. 2005)

- **In all 3 studies, standardized mortality ratios (SMRs) have been calculated for oral cavity/pharynx and cancers of the digestive organs**

Factors to Assess Risk of Bias	Study Details
Confounding and selection	Smoking data at the individual level (Birk et al. 2006, Luippold et al. 2005) Information collected on age (all studies) and race (Birk et al. 2006, Luippold et al. 2005)
Measurement error	Vital status and cause of death obtained for each cohort member (all studies) Cr(VI) exposure reconstructed for each cohort based on employment history (Birk et al. 2006)

Conclusions

- **Occupational cohort studies with individual-level data would be of far greater utility than ecological studies**
- **It is challenging to put together a comprehensive database of epidemiologic studies of Cr(VI).**
- **However, for purposes of evidence integration, the process really needs to include consideration/judging the strength of evidence, as recommended by NRC**
- **Good models to consider: The Cochrane Collaboration and their assessments which include a number of topics (e.g., cranberries and UTI, vitamin C and zinc and common cold)**
- **EPA should reissue the evidence tables before proceeding with risk assessment including analyses of risk of bias and generalizability**

Science Question 5: Cr-DNA Adducts

Key Points:

- **No empirical data support that Cr-DNA adducts occur *in vivo;* only mutagenic in highly contrived *in vitro* systems (Wise and Wise 2012; Thompson et al. 2013)**
- **Nuclear bioavailability of Cr(VI) is limited due to extracellular reduction and cytoplasmic trapping**
- **At Cr(VI) doses sufficient to damage DNA in mammalian cells, Cr(VI) is cytotoxic**
- **Current data do not support a role for Cr-DNA adducts in the MOA at know tumor sites (ingestion and inhalation)**

Deborah Proctor

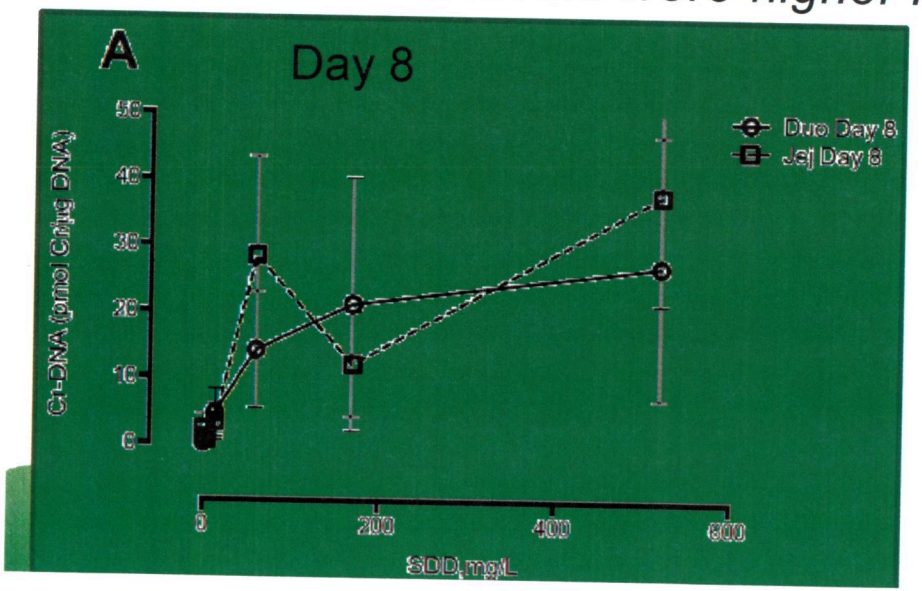
ToxStrategies, Inc.

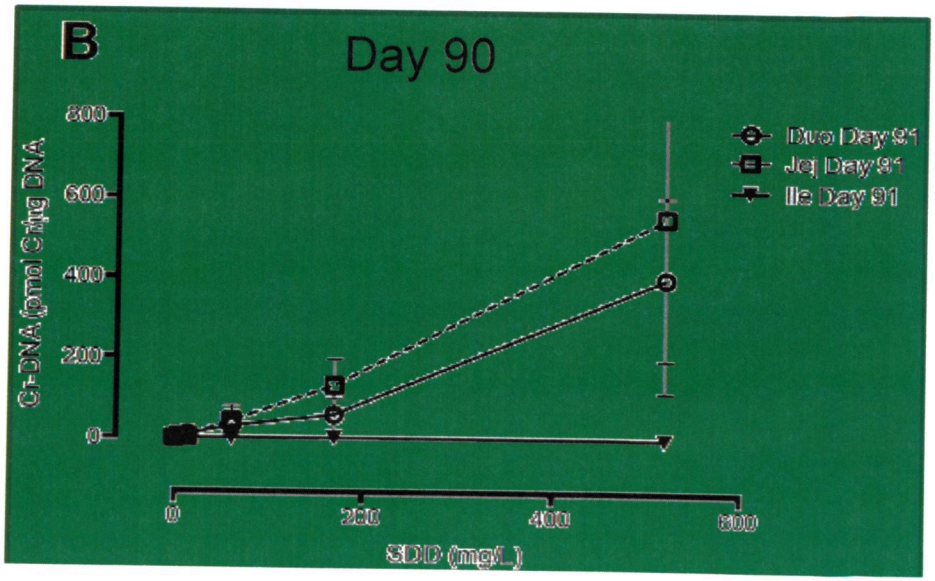
October 30, 2014

ToxStrategies

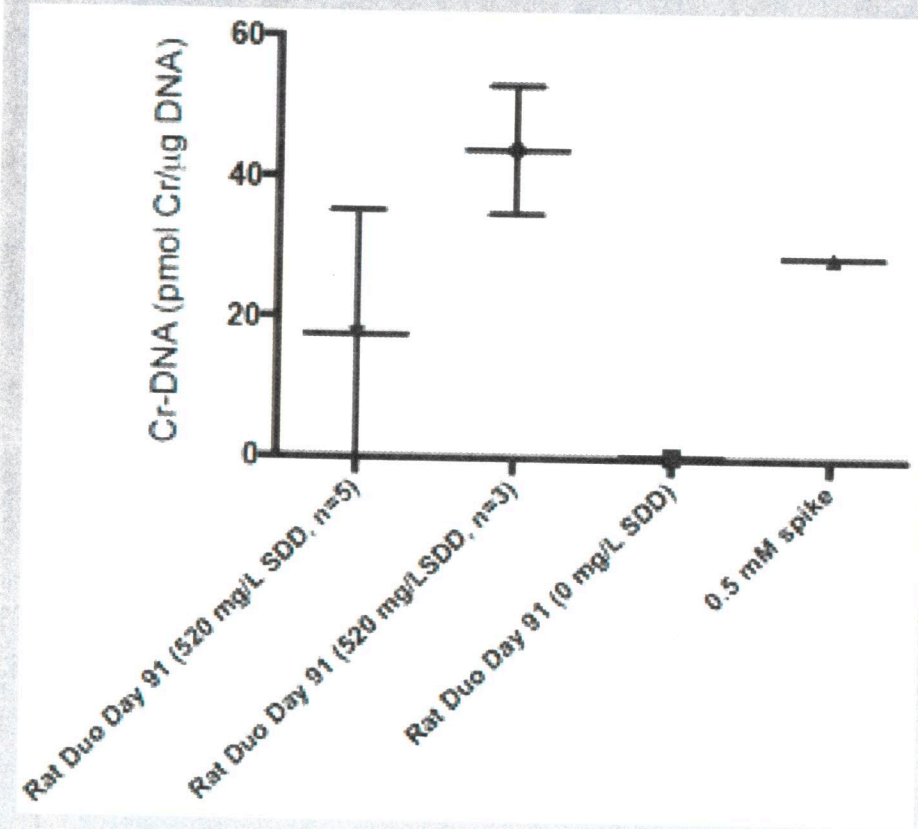
In Vivo Cr-DNA Binding (O'Brien et al. 2013-App B)

- **Collected Cr-DNA binding data *in vivo* in rat and mouse target tissues**
  - *Findings support results as biomarker of exposure*
  - *Current findings do not support a role for Cr-DNA adducts in the MOA for oral cavity and small intestine tumors*
- **Measured levels of Cr-DNA binding were not specific to responsive tissues**
  - *Cr-DNA binding was higher in the mouse jejunum than duodenum*
  - *Cr-DNA binding was increased in the mouse liver*
  - *Cr-DNA binding was elevated in the rat oral cavity at Day 8 than day 91 and levels were higher in the non-responsive mouse*





Cr-DNA Binding *In Vivo* Data Are Uncertain



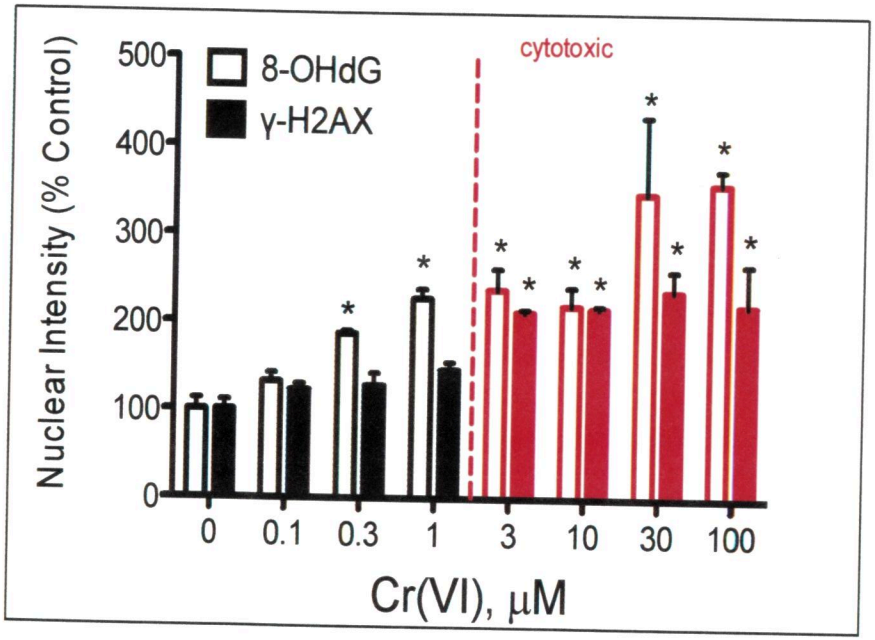
Source: O'Brien et al. (2013) Appendix B

Quality Control Assessments Demonstrates Problems

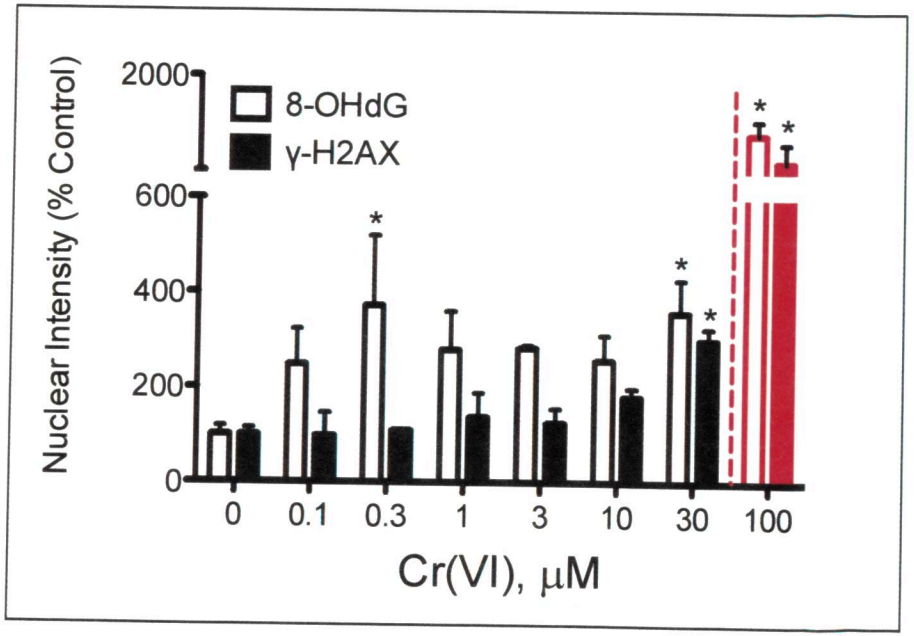
- Cr-DNA binding occurs *ex vivo* during digestion/DNA extraction
- Cr-DNA binding was not reproducible
  1. Two analyses of Cr-binding in rat duodenum at 520 mg SDD/L result in significantly different results
  2. Cr-DNA binding in Cr(III)-spiked control rat intestine sample demonstrated high levels of Cr-bound to DNA

Cr(VI) Double Strand Breaks Occur at Cytotoxic Doses

Undifferentiated CACO-2



Differentiated CACO-2



1. Cr(VI)-induced double strand breaks (DSB) occur at cytotoxic concentrations—high dose effect
2. Oxidative DNA damage is a more sensitive effect than DSB

Source: Thompson et al. 2012 *Plos One*

Cytotoxic Doses

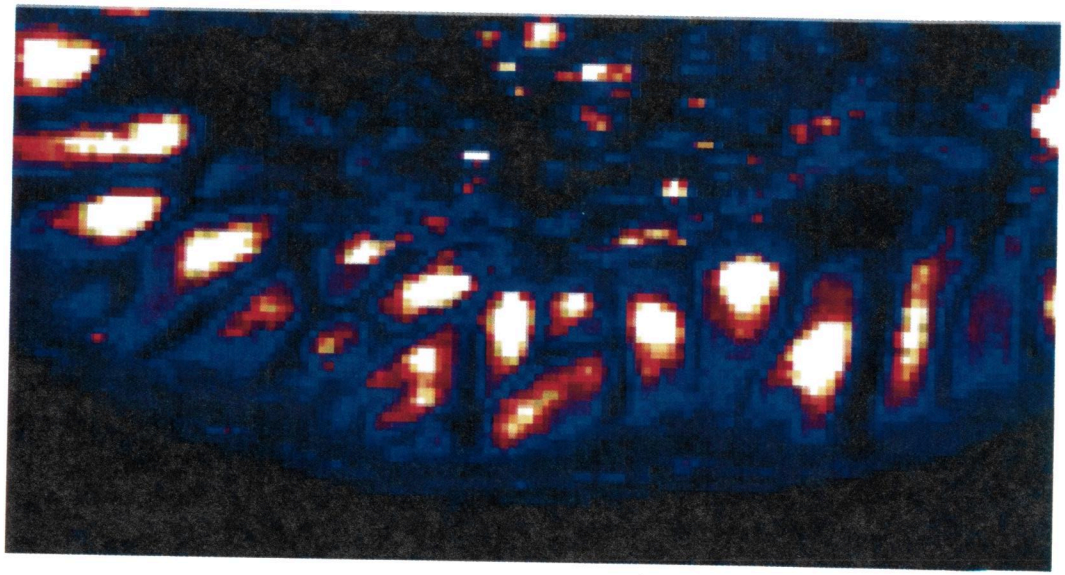
No Evidence of Cr-DNA Adducts or Mutations in Cr(VI) MOA based on *In Vivo* Data

MOA for Intestinal MOA

- Cr accumulated in mouse small intestinal villi, not crypts
- No increase in H2AX in intestinal crypts

(Thompson et al. accepted *Tox Sci*)

- No evidence of DNA damage or k-*ras* mutations in crypt (O'Brien et al. 2013)



MOA in Oral MOA

- No increase in mutations in rat oral cavity in Big Blue (In Review; as discussed for Question 7)

Role for Cr-DNA Adducts in Inhalation MOA is Not Supported by *In Vivo* Data

- **Cr-DNA adducts have not been reported *in vivo***
- ***In vitro* studies (e.g., Reynolds et al. 2004, 2007, and 2009) have shown that in human lung cells, Cr-DNA adduct is observed. However:**
  - Requires the use of plasmid vectors (Wise and Wise, 2012 referred to them as "experimentally contrived systems")
  - Supplementation with high levels of ascorbate (1.4 mM in Reynolds et al. 2007) is needed to from Cr-DNA adducts
  - Doses of Cr(VI) administered are cytotoxic. Cr-DNA adducts are observed when cell viability is low
- **Tumors in Cr(VI)-exposed workers show low P53 mutation frequency (Kondo et al. 1997)**
- **Animal data do not support mutagenic MOA in lung, oral cavity or intestine**
- **Overall, relevance of *in vitro* Cr-DNA adduct data is not supported based on the most recent MOA research data**

Bohn, Brent

From: Gibbons, Catherine
Sent: Friday, December 04, 2015 7:38 PM
To: Bohn, Brent
Subject: FW: News Update: NAS Readies EPA-Funded Reviews of IRIS Meeting Input, Human Studies (InsideEPA)

From: Gibbons, Catherine
Sent: Tuesday, October 21, 2014 10:25 AM
To: Elaine.Khan@oehha.ca.gov
Subject: FW: News Update: NAS Readies EPA-Funded Reviews of IRIS Meeting Input, Human Studies (InsideEPA)

Hi Elaine, thanks for your message...it wasn't in time to prevent this however! (see below highlighted) Not a big deal, but still.

From: Bland, Naseera
Sent: Monday, October 20, 2014 2:26 PM
To: Alcala, Cecilia; Alexander, Laurie; Avery, James; Ball, James; Bateson, Thomas; Berner, Ted; Birchfield, Norman; Bland, Naseera; Blessinger, Todd; Boone-Edwards, Amanda; Brinkerhoff, Chris; Buckley, Barbara; Burgoon, Lyle; Bussard, David; Cai, Christine; Carmichael, Brenda; Chiu, Weihsueh; Choudhury, Harlal; Christensen, Krista; Cogliano, Vincent; Corona, Elizabeth; Cubbison, Christopher; CURTIS, LUCY; D'Amico, Louis; Deener, Kathleen; Euling, Susan; Evans, Amanda; Field, Malcolm; Flowers, Lynn; Frederick, Bob; Frithsen, Jeff; Fritz, Jason; Galizia, Audrey; Gamble, Janet; Gatchett, Annette; Gehlhaus, Martin; Gibbons, Catherine; Glenn, Barbara; Grambsch, Anne; Gwinn, Maureen; Haque, Mefruz; Hawkins, Belinda; Hogan, Karen; Hotchkiss, Andrew; Iuliano, Kayla; Jarabek, Annie; Jinot, Jennifer; Johnson, Maureen; Jones, Samantha; Kadry, Abdel-Razak; Keshava, Nagalakshmi; Knecht, Helen; Kopylev, Leonid; Kraft, Andrew; Latham, Jessica; Lee, Janice; Lin, Yu-Sheng; Long, Tom; Luke, April; Makris, Susan; Marcus, Allan; Moore, Danielle; Murphy, Patricia; Nath, Raghu; Newhouse, Kathleen; Olden, Kenneth; Owens, Beth; Pardo, Larissa; Perovich, Gina; Persad, Amanda; Petersen, Dan; Powers, Christina; Pratt, Margaret; Preuss, Peter; Reid, Jon; Rieth, Susan; Ris, Charles; Ross, Christine; Ross, Mary; Rutigliano, Marian; Salazar, Matt; Sams, Reeder; Samuels, Crystal; Sanchez, Yolanda; Sasso, Alan; Schappelle, Seema; Schlosser, Paul; Scott, Cheryl; Segal, Deborah; Shams, Dahnish; Shaw, Denice; Slimak, Michael; Sonawane, Bob; Spassova, Maria; Strong, Jamie; Suter, Glenn; Taylor, DebraLynn; Troyer, Michael; Vandenberg, John; Vinikoor-Imler, Lisa; Vulimiri, Suryanarayana; Walker, Teneille; Walsh, Debra; Wang, Nina; White, Paul; Woodall, George; Wright, Barbara; Wright, Michael; Yang, Hui-Min; Zwayer, Bette
Subject: News Update: NAS Readies EPA-Funded Reviews of IRIS Meeting Input, Human Studies (InsideEPA)

FEDERAL FACILITIES WATCH

NAS Readies EPA-Funded Reviews Of IRIS Meeting Input, Human Studies

Posted: October 16, 2014

The National Academy of Sciences (NAS) is preparing to start new EPA-funded projects selecting speakers for the agency's public Integrated Risk Information System (IRIS) meetings that advocates say favor industry input too greatly, and assessing EPA's criticized practices for testing humans' reactions to exposure to contaminants.

The projects could, depending on their outcomes, potentially overhaul the lineup of speakers at the IRIS meetings and recommend changes EPA should make to its human testing procedures.

Starting in December 2012, EPA's IRIS program has instituted a practice of holding bimonthly public meetings to discuss ongoing assessments of chemicals, seeking scientific discussion relating to how to assess the chemicals' toxicity. But environmentalists have protested what they see as the meetings' imbalance, as most of the scientists who sign up to speak at the meetings are company scientists or industry consultants.

In an undated proposal, NAS explains that its staff will screen "candidate experts . . . for expertise relevant to the scientific and technical questions to be posed by EPA and for their availability to participate in the IRIS public science meetings. Information will also be gathered on possible conflict or bias issues and provided to EPA. If selected to participate in one of its IRIS public science meetings, each expert will speak on his or her own behalf."

That would be a marked contrast from previous IRIS bimonthly meetings, where most of the scientists who spoke did so on behalf of various companies or chemical industry groups.

Scientists with public health and advocacy groups boycotted a June IRIS meeting to protest the speakers for the arsenic and hexavalent chromium (Cr6) reviews discussed. All but two of the Cr6 speakers represented various industry groups, and many of the arsenic speakers were industry representatives.

IRIS Meeting

EPA's next IRIS bimonthly meeting, scheduled for Oct. 29-30 in Arlington, VA, appears to have a similar lineup of speakers. According to an agenda released Oct. 8, all of the scientists signed up to discuss a number of issues EPA has identified in its ongoing assessment of diisononyl phthalate are company scientists or industry consultants. Most represent the American Chemistry Council (ACC) or ExxonMobil Biomedical Sciences.

The agenda for the October meeting also includes discussion of the long-running assessment of Cr6, where all but one of the speakers represent industry, mostly ACC or the Electric Power Research Institute. The lone exception is David Ting, chief of California EPA's Pesticide and Environmental Toxicology Branch

"One critical aspect for the success of the EPA IRIS public science meetings is identifying the appropriate individuals to participate and provide advice on the various topics," NAS' proposal states. "Accordingly, EPA has asked the [NAS] to assist the agency with identifying candidates to participate in its IRIS public science meetings."

The proposal explains that NAS staff "will identify up to ten experts who could be invited to participate as individuals in the public science meetings. One expert may be international to cover a specific expertise not readily found in the U.S." Staff will apply NAS' policies on bias and conflict of interest when screening potential speakers and will consult with NAS' general counsel as part of the work.

The proposal adds that the NAS' National Research Council (NRC), whose staff will select the speakers, "is uniquely suited to provide such advice given its familiarity with IRIS issues and topics and its experience in assisting the Government Accountability Office (GAO) with a similar activity. Currently, NRC provides GAO with assistance in obtaining technical expertise that is required to provide reports to Congress on a wide array of topics."

Unlike many NAS projects for EPA, the proposal will not result in a written report. It will be done under a one-year $350,000 contract. At EPA's "discretion, this program may be extended for an additional two years," the proposal says.

Human Studies

NAS' other recently announced project is an 18-month, $820,000 effort by a committee to draft recommendations regarding EPA's conduct for controlled human exposure studies.

EPA's testing involving human exposure to pollutants has prompted criticism and litigation from some groups who fear harms to participants' health. A free-market group sued EPA to try and win a federal district court injunction barring the agency from continuing the tests and blocking any regulations based in part on the results of such testing. But the judge overseeing that suit dismissed it in January 2013, saying that the group lacked jurisdiction and standing to sue.

The agency's Office of Inspector General (IG) separately launched an investigation into a handful of the agency's human studies, each involving fine particulate matter or diesel exhaust. NAS' undated proposal indicates the committee will consider the IG's recommendations, released last March, as part of its work.

The IG concluded that EPA meets rules on human testing but should update its policies to better protect participants' health and inform them of the risks in studies. The report found that EPA followed applicable regulations when it exposed 81 subjects to concentrated airborne particles or diesel exhaust in five studies conducted during 2010 and 2011. The IG said EPA properly vetted the research plans through its review office, but the agency's guidance did not address when researchers are required to seek review again if there are significant modifications to the study.

While consent forms for the tests met applicable regulatory requirements, they did not always consistently represent the exposure risks subjects might face. EPA should also make clear in its policies, guidance and consent forms its responsibilities for clinical follow-up of any adverse health events that occur with the tests, the IG said.

EPA responded to the IG's findings by vowing to improve its policies to ensure protection of humans involved in scientific testing. Acting EPA research chief Lek Kadeli said the agency will revise its internal guidance to address significant modifications to studies; has already implemented a procedure to document that investigators overseeing human subjects studies meet requirements for continuing ethics education; and will take steps to better ensure that guidelines on human testing are adhered to in the future.

NAS' undated proposal says the IG's "report has led to questions about EPA's conduct of human research studies and whether the agency should continue conducting such research."

EPA's Research

The proposal adds, "Because some of EPA's research was designed to address recommendations from previous [NRC] reports (NRC 1998, 1999, 2001, 2004) that recommended research priorities for the study of airborne particulate matter, EPA has asked the NRC to evaluate the degree to which EPA's studies of human subjects have been valuable to inform and reduce uncertainties in setting pollutant standards. If the committee's findings generally support the continuation of such research, EPA has requested guidance on how to improve the conduct of such studies to ensure they are approved, designed, and conducted consistently and ethically."

The committee will weigh in on issues such as whether EPA's human studies have "been valuable to inform and reduce uncertainties in setting pollutant standards? Is it warranted to continue to conduct controlled human-exposure studies as part of EPA's larger research agenda for air pollutants?" The committee is also asked to "assess the potential health risks to test subjects who participated in recent studies of air pollutants at EPA's clinical research facility and comment on the degree of actual risk imposed by the exposures in those studies."

If the committee supports EPA's continuation of such studies, it will provide recommendations on "[m]ethods for estimating levels of risk in controlled human-exposure studies, drawing from relevant approaches used in Phase I clinical drug trials." It would also provide a template for the agency in future studies to "characterize reasonably foreseeable risks, in terms of the nature, frequency, and magnitude of possible risks, which could be used in obtaining informed consent from potential study participants." -- *Maria Hegstad (mhegstad@iwpnews.com)*

Naseera H. Bland
Science Communications Team Contractor
National Center for Environmental Assessment
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O: 703.347.0402
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Bohn, Brent

From: Gibbons, Catherine
Sent: Friday, December 04, 2015 7:36 PM
To: Bohn, Brent
Subject: FW: Cr(VI) bimonthly presentations
Attachments: 7_Young_Chromium Science Question 7 Young 22Oct14.pptx; 8_Thompson_Thompson_Cr_8_Oct.pptx; Is_Cr(VI)-Induced_Mutagenesis_Essential.pptx; The_importance_of_early_consideration_of_Mode_of_Action.pptx; 3_Hays_EPA Cr Science Meeting 103014 Q3_Part 1.pptx; 3_Kirman_EPA Cr Science Meeting 103014 Q3_Part 2.pptx; 4_Harris_Harris Science Question 4.pptx; 4_Thompson_Thompson_Cr_4_Oct.pptx; 6_Thompson_Thompson_Cr_6_Oct.pptx; 7_Thompson_Thompson_Cr_7_Oct_revised.pptx

From: Gibbons, Catherine
Sent: Friday, October 24, 2014 3:22 PM
To: Elaine.Khan@oehha.ca.gov
Subject: Cr(VI) bimonthly presentations

Greetings! These are all publicly available on the docket, so I thought I'd save you the trouble and send them along. Enjoy!

--Catherine

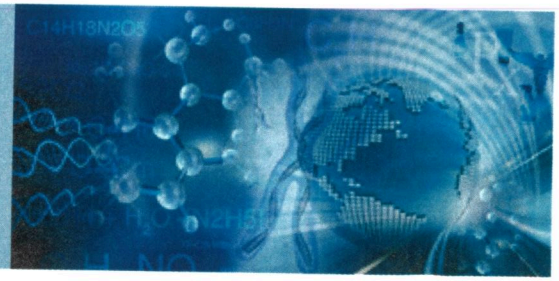
Integrated Risk Information System (IRIS) Bimonthly Public Science Meeting
Thursday, October 30, 2014

Science Question 7: *In vivo* mutagenicity/genotoxicity studies of hexavalent chromium

Robert Young
BioReliance
Rockville, Maryland

BioReliance®
by SAFC

Big Blue® Transgenic Rodent (TGR) Mutation Assay



- *In vivo* assay to measure somatic and germ cell mutations
- Historically – limited options to measure *in vivo* mutations
- TGR Mutation assays developed and validated in 1990's
- Filled an unmet need to investigate *in vivo* mutagenic mode of action
- Uses transgenic mice and rats with recoverable lambda shuttle vector

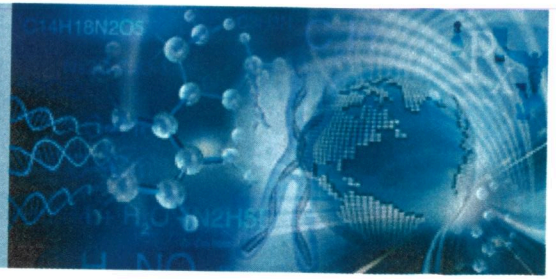
Big Blue® Assay History

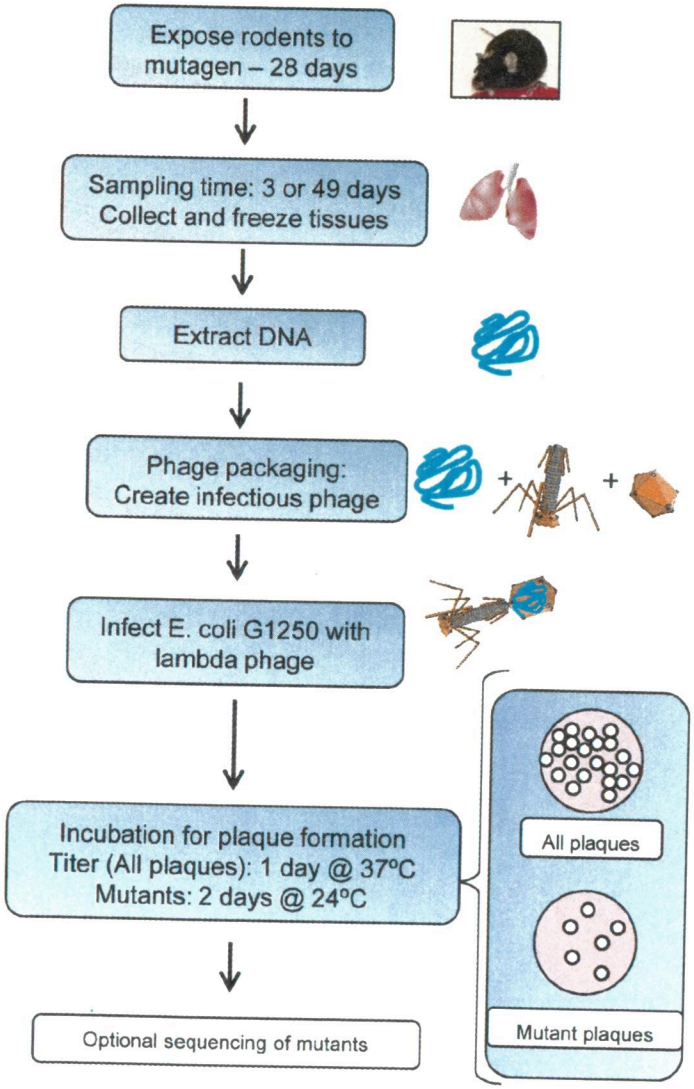
- NTP contracted BioReliance to create TGR mutation models
- BioReliance validated and commercialized the assays in 1990's
  - In absence of guidelines, little commercial interest through 2000's
- OECD Technical Guideline 488 in 2011 reawakened interest and use
  - Used for ECHA, EFSA, pharmaceuticals (actives and impurities)
- BioReliance owns Big Blue® mice and rats
- BioReliance re-qualified Big Blue® to new OECD design standards

Big Blue® Assay History

- NTP goal – use same species/strains used by NTP for 2 year bioassays.
- Original purpose to investigate tumor MOA from NTP cancer studies
- Rat:
  - Big Blue® Fisher 344 rat; Homozygous
  - F344 rat used for NTP 2 year rat carcinogenicity studies
- Mouse:
  - Created in Big Blue® C57BL/6 mice; Homozygous
  - Breed to C3H mice to create Big Blue® B6C3F1 mice; Heterozygous
  - B6C3F1 used for NTP 2 year mouse carcinogenicity studies

Big Blue® Assay: Overview





- Dose animals
- Necropsy - freeze tissues
- Extract DNA
- Cut out shuttle vector (Transpack)
- Package into empty phage particles
- Adsorb onto *E. coli* G1250
- Plate onto 100 mm plates
- Incubate at 37°C and 24°C
  - 37°C – both *cII* wildtype and mutants give plaques
  - 24°C – only *cII* mutants produce plaques
- Count and evaluate
- Mutant frequency: ratio of mutants to total phage (plaques) screened

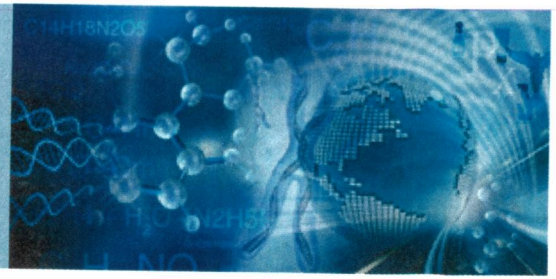
BioReliance® by SAFC

Modifications to Analyze Mutations in Oral Cavity

- Oral cavity not routinely evaluated in TGR assays
- Standard methods gave low yield of low quality DNA
- Methods optimized in two studies
  - Liquid nitrogen pulverization of tissue
  - Homogenization, centrifugation of nuclei, digestion, phenol chloroform extraction

BioReliance® by SAFC

Oral Mutagenesis Proof of Concept: Gingiva - Buccal

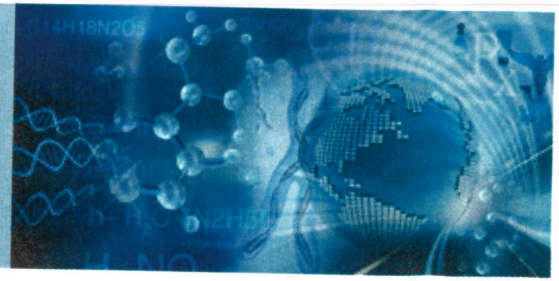


Treatment (mg/kg/day) x days	Animal Number	# Packaging	# Mutants	# Phage Screened	Mutant Frequency ($x10^{-6}$)
Drinking water (0.00) x 28	2451	2	21	289,333	72.6
	2452	2	12	211,000	56.9
	2453	4	11	169,667	64.8
	2454	4	6	174,333	34.4
	2455	2	5	184,000	45.7
	Average ± Standard Dev	---	---	---	**51.2 ± 19.6**
4-NQO (10 ppm) x 28	2456	2	286	252,333	1133
	2457	2	283	214,333	1320
	2458	2	213	204,333	1042
	2459	4	207	220,333	939
	2460	3	137	134,333	
	Average ± Standard Dev	---	---	---	**1091* ± 146**

***Significant increase ($p < 0.001$)**

BioReliance® by SAFC

Oral Mutagenesis Proof of Concept: Gingiva - Palate

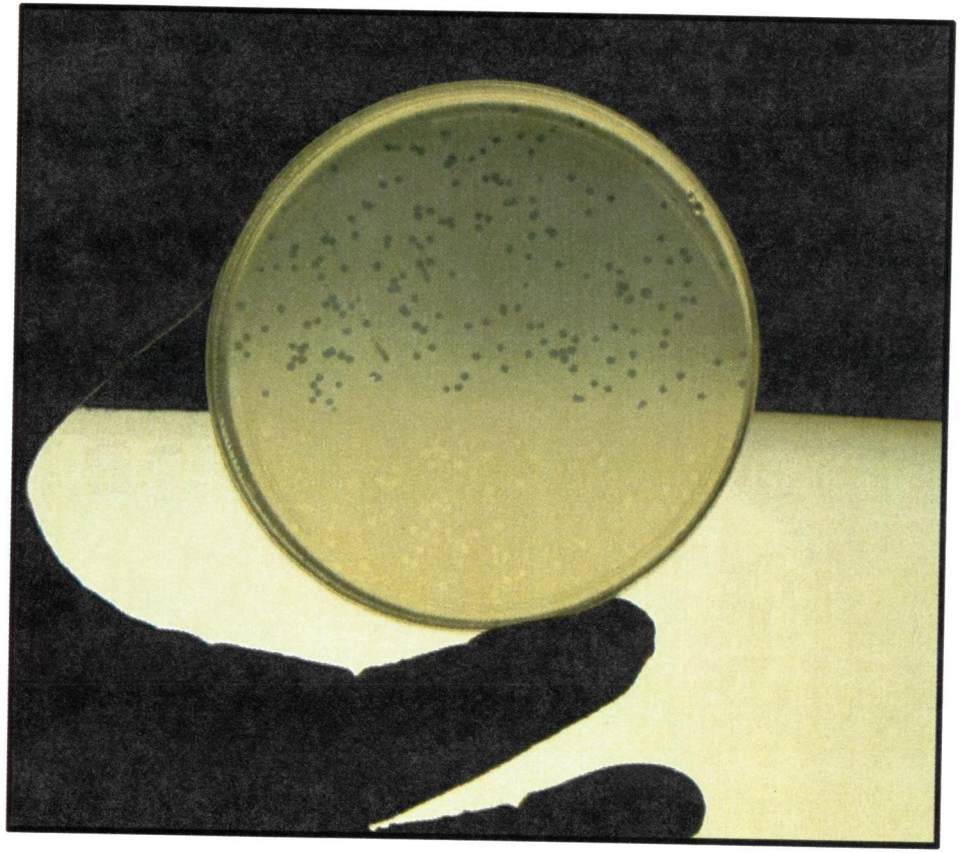


Treatment (mg/kg/day) x days	Animal Number	# Packaging	# Mutants	# Phage Screened	Mutant Frequency ($x10^{-6}$)
Drinking water (0.00) x 28	2451	2	7	213,333	32.8
	2452	2	16	329,333	48.6
	2453	2	12	215,000	55.8
	2454	2	15	315,667	47.5
	2455	3	13	315,667	41.2
	Average ± Standard Dev	---	---	---	**45.2 ± 8.6**
4-NQO (10 ppm) x 28	2456	2	439	203,667	2155
	2457	4	619	277,333	2232
	2458	2	682	364,667	1870
	2459	3	584	286,000	2042
	2460	2	634	207,667	3053
	Average ± Standard Dev	---	---	---	**2271* ± 458**

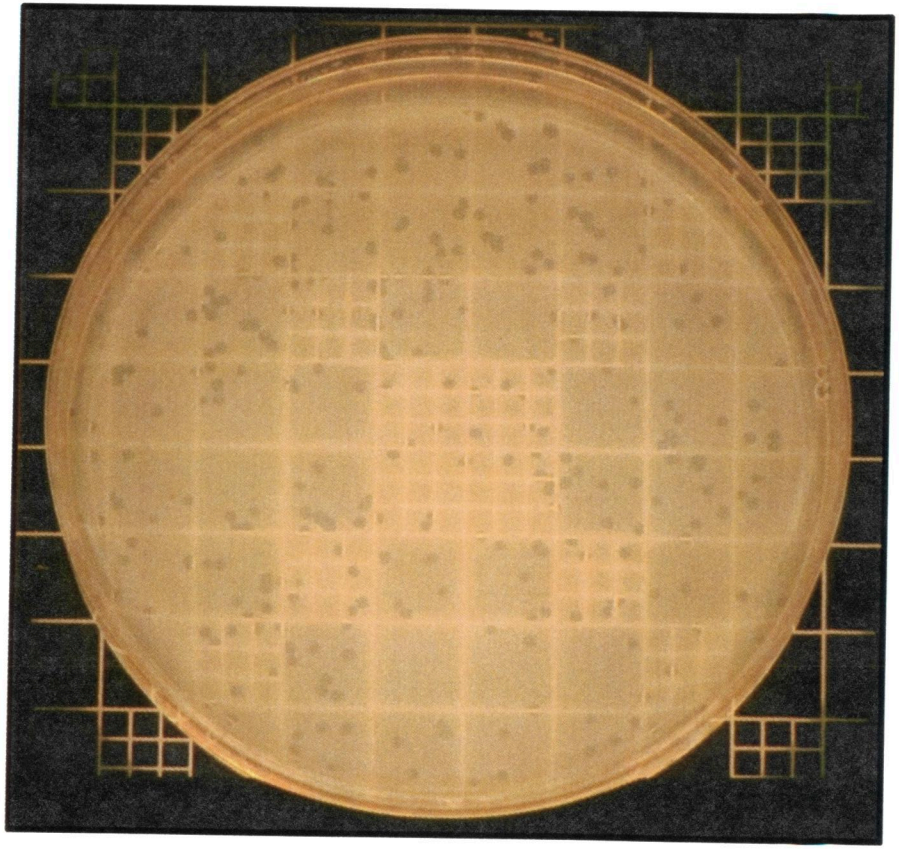
***Significant increase ($p < 0.001$)**

BioReliance by SAFC

Big Blue® in Pictures



E. coli lawn with plaques



Scoring plaques

BioReliance® by SAFC

Cr(VI) in the Big Blue® Assay

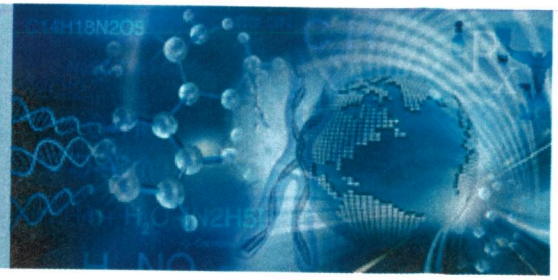


FIGURE IN PREPARATION

ToxStrategies

BioReliance® by SAFC

Science Question 8: Definitions

Chad Thompson, Ph.D.
ToxStrategies, Inc.
Supported by ACC
Oct 30, 2014

ToxStrategies

Definitions of Genotoxicity & Mutagenicity

For this assessment, the IRIS Program is considering using the following definitions found in the EU Technical Guidance on Risk Assessment (1996)…

Comments:

- **What is meant by "for this assessment"?**
  - **Do the definitions differ across assessments?**
  - **Does EPA intend to adopt these definitions formally?**
  - **Has EPA considered whether these definitions conflict with *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005)**
- **The proposed definitions can be found in Section 3.10 of EU guidance document (2003)*, which contains important information and recommendations for considering the genotoxicity and mutagenicity of a chemical…**

*European Commission. (2003) Technical guidance document in support of commission directive 93/67/EEC on risk assessment for new notified substances and commission regulation (EC) No 1488/94 on risk assessment for existing substances, Part I.

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Section 3.10: EU 2003 Guidance Document*

1. "Evaluation of genotoxicity test data should be made with care. Regarding 'positive' findings, responses may be generated only at highly toxic/cytotoxic concentrations, and the presence or absence of a dose-response relationship should be considered."
2. "In vitro tests are particularly useful for gaining an understanding of the potential mutagenicity of a substance…Animal tests will…be needed, however, for the clarification of positive findings…"
3. "Following a positive result in an in vitro mammalian cell mutagenicity test, adequately conducted somatic cell in vivo testing is required to ascertain if this potential can be expressed in vivo."
4. "Select adequate somatic cell in vivo test, primarily on basis of systemic availability of the test substance:
   1. adequate systemic availability:
   - Micronucleus test (pref. for in-vitro clastogens and/or aneugens)

   2. lack of adequate systemic availability:
   - studies with tissues at initial sites of contact, e.g. in vivo comet assay; gene mutation with transgenic mice"

Can also do MN in GI tissue

3

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γ-H2AX staining and MN in duodenum (O'Brien et al., 2013)

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Can also do MN in GI tissue

Big Blue rat OECD guideline study in rat oral mucosa

γ-H2AX staining and MN in duodenum (O'Brien et al., 2013)

In vivo kras mutation in duodenum (O'Brien et al., 2013)

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ToxStrategies

Is Cr(VI)-Induced Mutagenesis Essential and therefore a KE in the MOA for small intestinal tumors in mice? Hyperplasia?

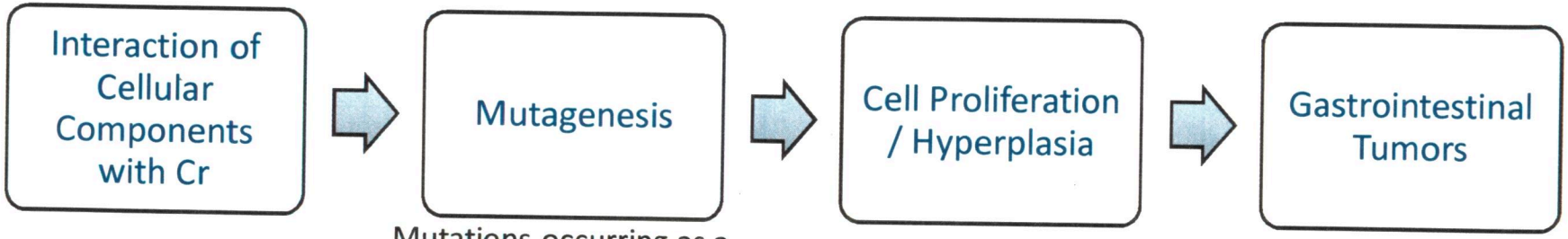
Essentiality of Key Event	Defining Question	High (Strong)	Moderate	Low (Weak)
	Can the KE be shown to occur in the affected tissue or organ? Are downstream key events and/or the critical effect prevented if an upstream key event is blocked? [e.g., stop/reversibility studies, antagonism, knock out models, etc.)	Multiple lines of experimental evidence illustrating essentiality for several of the key events	There is at least one line of experimental evidence indicating essentiality of an important key event	Indirect or no experimental evidence of the essentiality of any of the key events

Genotoxic is not the same as mutagenic. Standard genotoxicity assays were not designed to inform specific modes of tumor induction. ... these other assays (non-mutagenic assays) do not measure heritable events, but rather measure evidence of DNA damage or its repair. Non-mutagenic assays include chromosome aberrations, micronuclei, comet assays, DNA lesion measurements, and DNA repair assays. ... They provide only supportive evidence that mutagenesis might be a MOA. DNA damage per se does not inform us about eventual heritable change, which is the true issue. Most (but not all) mutagens cause heritable changes in DNA sequences by causing damage to DNA (pre-mutagenic lesions) that is converted to mutation after cell division.[4]

- Professor Toby Rossman, 2010 IRIS Cr(VI) Peer Review

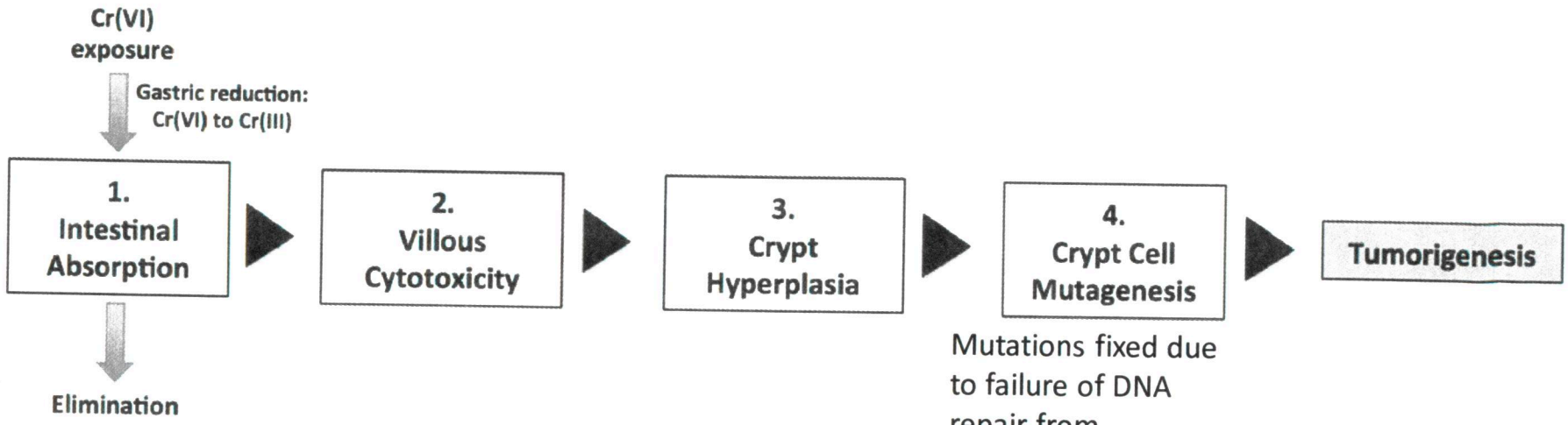
Two Competing MOA Hypotheses

McCarroll et al. (2010)[2]



Mutations occurring as a direct result of Cr-induced DNA damage

Thompson et al. (2013)[3]



Mutations fixed due to failure of DNA repair from shortened cell cycle

Evidence Integration Including evidence of DNA Damage or Mutation

- Supporting Evidence / Damage
  - Point mutations in bacteria (Ames Test)[2]
  - DNA damage in liver, WBCs, brain, skin and bone marrow of mice[2]
  - Increases in 8-OHdG and DNA-protein crosslinks (DPXL) in isolated mouse duodenal cells treated for 1 hr in vitro[5]
- Supporting Evidence / Mutation
  - Positive mammalian spot test (coat color change in offspring) in mice exposed to welding fumes containing chromate[6]
  - Mutations in bone marrow and liver of transgenic mice exposed to a single concentration of Cr(VI) measured by packaging into a phage, infection of E. coli and occurrence of mutant plaques[7]

- Refuting Evidence / Damage
  - Lack of any dose-related change in 8-OHdG adducts in duodenal epithelium of mice treated for 3 months[8]
  - No change in 8-OHdG adducts or DPXL in mouse duodenal epithelium after 9 months of drinking water exposure to 5 or 20 mg/L Cr(V)) for 9 months[5]
- Refuting Evidence / Mutation
  - Lack of dose-related effect of K-Ras codon 12 GGT-GAT mutation in mouse duodenal epithelium[9]
  - The genomic signature and expression of 4 genes involved in the DNA damage response induced by Cr(VI) in mouse duodenal epithelium were much more similar to those of non-mutagenic carcinogens than to mutagenic carcinogens[10]

Duodenal Epithelium of Mice

Why Including these Papers is Critical?

- If a mutagenic MOA is assumed, linear low-dose extrapolation along with an age-dependent adjustment factors will result in an overestimate of Cr(VI) carcinogenicity.
- Assuming a non-mutagenic MOA will likely result in choosing a precursor or sentinel key event and the use of non-linear low-dose extrapolation resulting in an RfD protective of cancer, similar to that for chloroform. (p. 32-33 of NAS Review)
- The Arsenic preliminary materials provide a starting point for MOA consideration.

The Importance of Early Consideration of Mode of Action

Ted Simon, Ph.D., DABT

Comments to

Docket EPA-HQ-ORD-2014-0313

IRIS Systematic Review from NAS 2014

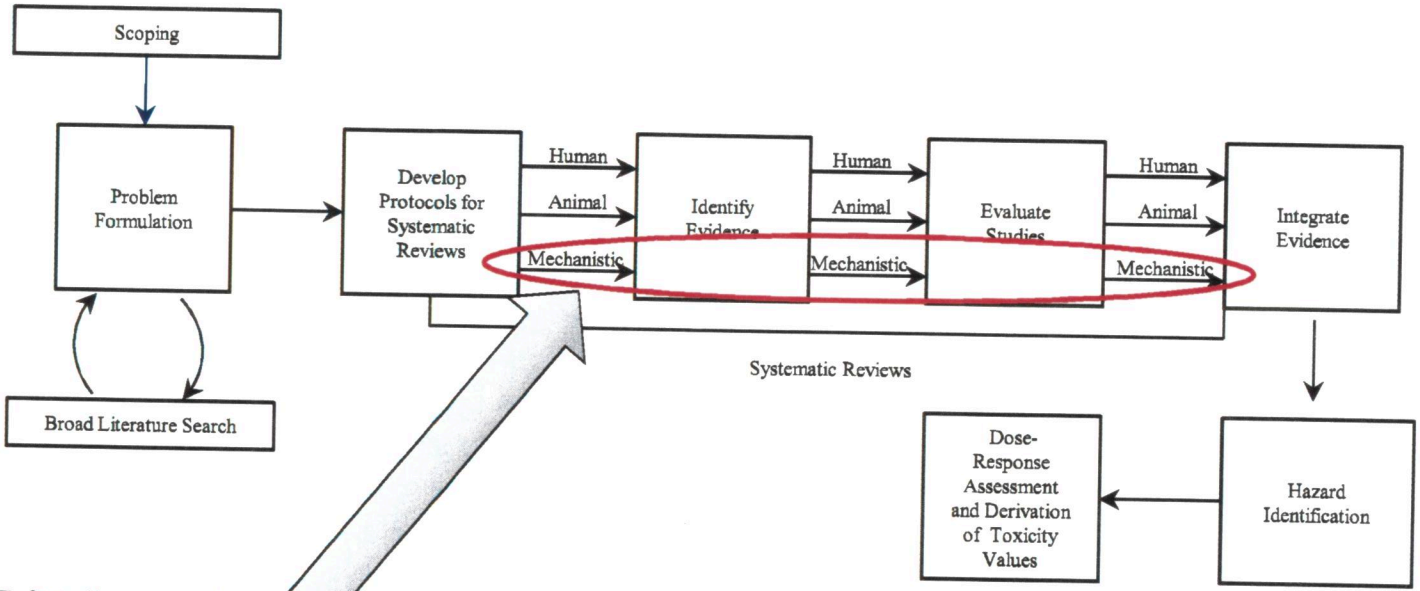


FIGURE S-1 Systematic re in the context of the IRIS process. The committee views public input and peer review as integral parts of IRIS process, although they are not specifically noted in the figure.

"Mechanistic" refers to MOA. To identify evidence, there need to be hypothesized MOAs developed as part of protocol development for systematic review.[1]

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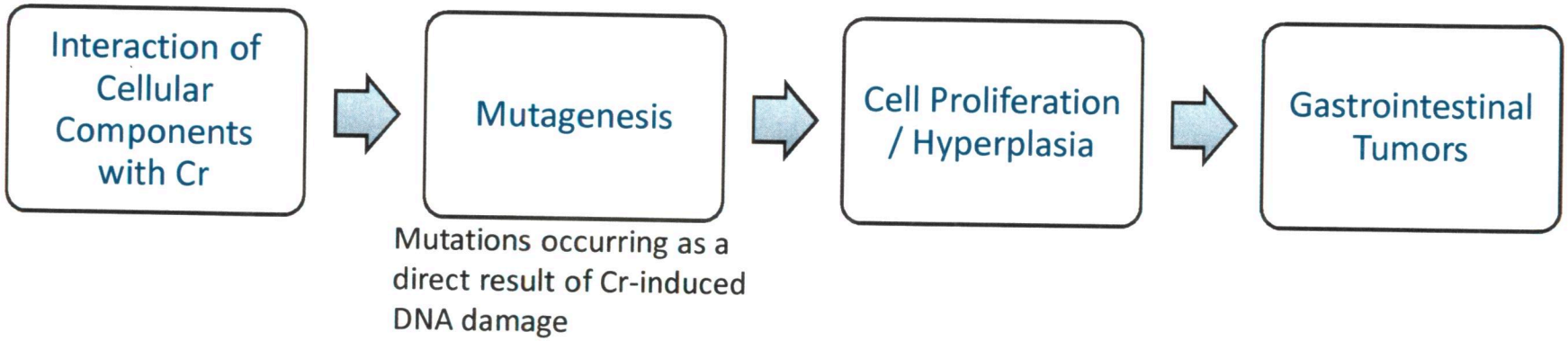

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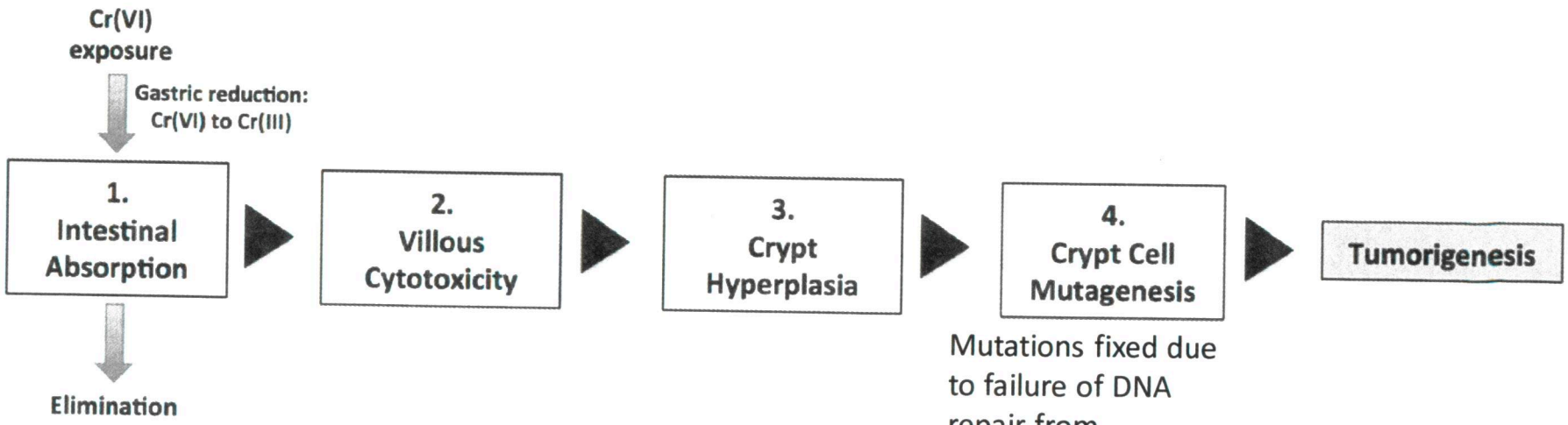

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Duodenal Epithelium of Mice

Excluding these seven studies was inconsistent with NAS 2014 Review

Study	Search Tags	Reason for inclusion
2. McCarroll et al. Environ Mol Mutagen. 2010, Mar;51(2):89-111.	Secondary Sources of Health Effects Data; Reviews	Presents a hypothesized MOA
3. Thompson CM, et al. Crit Rev Toxicol. 2013, Mar;43(3):244-74	Secondary Sources of Health Effects; Reviews; Jan to June 2013	Presents a hypothesized MOA
5. De Flora et al. Mutat Res. 2008, Jul;659(1-2):60-7	Secondary Sources of Health Effects; Reviews	Direct evidence relating to DNA damage in the target tissue
6. Knudsen I. Acta Pharmacol Toxicol (Copenh). 1980, Jul;47(1):66-70	Supporting Studies; Genotoxicity Studies	Direct evidence relating to Cr(VI) mutagenicity
7. Itoh S, and Shimada H. Mutat Res. 1998, Jan 13;412(1):63-7	Supporting Studies; Genotoxicity Studies	Direct evidence relating to Cr(VI) mutagenicity
8. Thompson CM et al. Toxicol Sci. 2011, Jun 28;123(1):58-70	Primary Sources of Health Effects Data; Animal Studies; Cited in Document (but not the DNA damage data)	Direct evidence relating to DNA damage in the target tissue
9. O'Brien TJ et al. Mutat Res. 2013, Apr 9;754(1-2):15-21.	Supporting Studies; Genotoxicity Studies; Jan to June 2013; June 2013 – Jan 2014	Direct evidence relating to Cr(VI) mutagenicity in the target tissue
10. Thompson et al. Regul Toxicol Pharmacol. 2012, Jun 15;64(1):68-76.	Supporting Studies; Genotoxicity Studies	Indirect evidence relating to Cr(VI) mutagenicity in the target tissue

Including these Papers is Critical! Why?

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- The Arsenic preliminary materials provide a starting point for MOA consideration.

Risk of Bias in Mechanistic Studies

- This is an area where the IRIS program will likely be a pioneer
- Table of Evidence Integration for MOA provided along with these slides
- Other resources—Arsenic Preliminary Materials[11]
- P 70-71 in the NAS Review[1].

Conclusions—A Plea for Early Consideration of MOA

- P. 82 of NAS Review
  - "One option is to organize the evidence around potential mechanisms by which a chemical might cause harm."
- Table 6-1 of the NAS Review provides a way to consider mechanistic information
- NAS 2014 Critical Aspects of Arsenic[12] document has general guidance for consideration of MOA
- Arsenic Preliminary Materials tried to include MOA
- As part of extra information I've provided, there's a table that uses the evolved Bradford Hill considerations to evaluate a hypothesized MOA[13] (Meek et al. 2014, J Appl Toxicol 34(6): 595-606)

References

1. National Research Council (NRC) (2014) Review of EPA's Integrated Risk Information System (IRIS) Process, p. 4 at http://www.nap.edu/catalog.php?record_id=18764
2. McCarroll N, Keshava N, Chen J, Akerman G, Kligerman A, and Rinde E. An evaluation of the mode of action framework for mutagenic carcinogens case study II: chromium (VI). Environ Mol Mutagen. 2010, Mar;51(2):89-111
3. Thompson CM, Proctor DM, Suh M, Haws LC, Kirman CR, and Harris MA. Assessment of the mode of action underlying development of rodent small intestinal tumors following oral exposure to hexavalent chromium and relevance to humans. Crit Rev Toxicol. 2013, Mar;43(3):244-74
4. EPA (2011) Peer Review of EPA's Draft Toxicological Review of Hexavalent Chromium, Post-Meeting Comments, July 6, p. 61 at http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=221433
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6. Knudsen I. The mammalian spot test and its use for the testing of potential carcinogenicity of welding fume particles and hexavalent chromium. Acta Pharmacol Toxicol (Copenh). 1980, Jul;47(1):66-70
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8. Thompson CM, Proctor DM, Haws LC, Hebert CD, Grimes SD, Shertzer HG, et al. Investigation of the Mode of Action Underlying the Tumorigenic Response Induced in B6C3F1 Mice Exposed Orally to Hexavalent Chromium. Toxicol Sci. 2011, Jun 28;123(1):58-70
9. O'Brien TJ, Ding H, Suh M, Thompson CM, Parsons BL, Harris MA, et al. Assessment of K-Ras mutant frequency and micronucleus incidence in the mouse duodenum following 90-days of exposure to Cr(VI) in drinking water. Mutat Res. 2013, Apr 9;754(1-2):15-21.
10. Thompson CM, Gregory Hixon J, Proctor DM, Haws LC, Suh M, Urban JD, and Harris MA. Assessment of genotoxic potential of Cr(VI) in the mouse duodenum: An in silico comparison with mutagenic and nonmutagenic carcinogens across tissues. Regul Toxicol Pharmacol. 2012, Jun 15;64(1):68-76.
11. EPA, Draft Development Materials for the Integrated Risk Information System (IRIS) Toxicological Review of Inorganic Arsenic. April 2014.
12. National Research Council (2014) Critical Aspects of EPA's IRIS Assessment of Inorganic Arsenic: Interim Report
13. Meek MEB, Palermo CM, Bachman AN, North CM, and Jeffrey Lewis R. (2014) Mode of action human relevance (species concordance) framework: Evolution of the Bradford Hill considerations and comparative analysis of weight of evidence. J Appl Toxicol 34(6):595-606.